

**Amendments to the Specification:**

Please insert the enclosed paper copy of the sequence listing into the application after the section entitled "Abstract" at the end of the application.

Please replace paragraph [0015] on page 4 of the application with the following amended paragraph:

[0015] Fig. 1A depicts the A-1 interaction. Pre-tRNA<sup>Archeuka</sup> (molecule 4) is provided as SEQ ID NO:72 in the sequence listing. Fig. 1B depicts a substrate cleaved in both the mature-domain dependent and the mature-domain independent modes.

Please replace paragraph [0019] bridging pages 4 and 5 of the application with the following amended paragraph:

[0019] Fig. 5 describes a scheme of BHB (bulge-helix-bulge) insertions in the GFP mRNAs. The upper and lower parts of the figure show the structure of the GFPof and the GFP-BHB genes, as well as the BHB position. In the middle, the different BHB substrates are detailed. The BHB (provided as SEQ ID NO:73 in the sequence listing) and the BHB-stop (provided as SEQ ID NO:74 in the sequence listing) are processed by the MJ-endoribonuclease whereas the BHB+3 (provided as SEQ ID NO:75 in the sequence listing) is MJ-endoribonuclease insensitive. Intronic ribonucleotides are underlined, stop codons blocking all reading frames are in bold face and the base-pair insertion disrupting the canonical BHB structure is boxed in green.

Please replace paragraph [0022] on page 5 of the application with the following amended paragraph:

[0022] Fig. 8 is a sequence analysis of RT-PCR products derived from NIH3T3 cells transiently transfected with pGFPof and pMJ plasmids. Sequences were performed using RT-PCR products eluted from a gel. In the middle, the arrows indicate the intron-flanking nucleotides. The top spliced sequence, the middle intron sequence, and the bottom unspliced sequence are provided as SEQ ID NO:76, SEQ ID NO:77, and SEQ ID NO:78 in the sequence listing, respectively.

Please replace paragraph [0024] on page 6 of the application with the following amended paragraph:

[0024] Fig. 10 is a sequence analysis of RT-PCR products derived from NIH3T3 cells transiently transfected with pGF-BHB and pMJ plasmids. Sequences were performed using RT-PCR products eluted from a gel. In the middle, the arrows indicate the intron-flanking nucleotides. The top spliced sequence, the middle intron sequence, and the bottom unspliced sequence are provided as SEQ ID NO:79, SEQ ID NO:80, and SEQ ID NO:81 in the sequence listing, respectively.

Please replace paragraph [0025] on page 6 of the application with the following amended paragraph:

[0025] Fig. 11 is a scheme of BHB insertions in reporter mRNAs: (A) the different BHB substrates are detailed. The BHB (provided as SEQ ID NO:73 in the sequence listing) is processed by the MJ endoribonuclease whereas the BHB+3 (provided as SEQ ID NO:75 in the sequence listing) is not. The BHB-stop structure (provided as SEQ ID NO:74 in the sequence listing) contains an intron in which three stop codons, blocking all reading frames, are inserted. (B) structures of the GFP-BHB, GFPof and BetaGALof genes, showing the positions of the BHB and the RT-PCR primers used for the experiments shown in (C). (C) splicing analysis of mRNAs derived from NIH3T3 cells transfected with plasmids coding for MJ endonuclease and pGFP-BHB or pGFP-BHB+3 target constructs. The figure shows RT-PCR analysis of different GFP mRNAs in the presence or absence of a functional MJ enzyme.

Please replace paragraph [0028] bridging pages 6 and 7 of the application with the following amended paragraph:

[0028] Fig. 14 demonstrates that a defective firefly luciferase mRNA is repaired by BHB-mediated trans-splicing: (A) Targeting and target RNAs. Target RNA (provided as SEQ ID NO:84 in the sequence listing) produces, with Targeting 1 RNA (provided as SEQ ID NO:82 in the sequence listing), a bona fide BHB, with Targeting 2 RNA (provided as SEQ ID NO:83 in the sequence listing) a classical double-stranded RNA. Targeting 1 and Targeting 2 RNAs differ by a total of six nucleotides (in boxes). Both Targeting 1 and Targeting 2 RNAs were transcribed from a CMV immediate early promoter; target RNA was transcribed from a SV40 promoter. (B) RT-PCR analysis shows that both possible recombinant RNAs are generated by the trans-splicing event. (C) Trans-activation of firefly luciferase requires both a bona fide BHB and an optimized MJ endonuclease.

Please replace paragraph [0030] on page 7 of the application with the following amended paragraph:

[0030] Fig. 16 is a functional analysis of the archaeal tRNA endonuclease obtained from the transgenic mouse (Fig. 16B) and structure and sequence (provided as SEQ ID NO:85 in the sequence listing) of the substrate utilized in the assay (Fig. 16A).

Please replace Table 1 on page 8 of the application with the following amended Table 1:

<b>TABLE 1</b>
<b>Oligodeoxyribonucleotides</b>
<p>P1- GAGCTCAAGCTTCGAATTCCTCCGGTCGTGACTCCAGAGGCTTACACCGGAGATATCAGACCGGTTGTGAGCAAGGGCGAG (SEQ ID NO:1)</p> <p>P2-ATCACGAGATCTCCACCATGGACTACAAAGACGATGACGATAAACTCGAGCTCAAGCTTCGAATT (SEQ ID NO:2)</p> <p>P3-TCGGGATCCTCTACAAATGTGGTATGGCTG (SEQ ID NO:3)</p> <p>P4-GCTTCGAATTCCTCCGGTCGTGACTTCTCCAGAGGCTTACACCGGAGAAGATATCAGACCGGTTGTGA (SEQ ID NO:4)</p> <p>P5-GCTTCGAATTCCTCCGGTCGTGACTCCAGAGGTAAGTAAACCGGAGATATCAGACCGGTTGTGA (SEQ ID NO:5)</p> <p>P6-ATAAGAATGCGGCCCGCGGTCGTGATATCTCCGGTGTAAGCCTCTGGAGTCACGACCGGGGACGGG (SEQ ID NO:6)</p> <p>P7-ATAAGAATGCGGCCCGCGGTCGTGATATCTCTCCGGTGTAAGCCTCTGGAGAAGTCACGACCGGGGACGGG (SEQ ID NO:7)</p> <p>P8-TCACGACCGGGGACGGGGGCCAGACGGAGGGCGAGTCCTTGAGCGCATCTACACATTGATCCTAGCAGA (SEQ ID NO:8)</p> <p>P9-CGTGAGATCCGCTAGCGCTAC (SEQ ID NO:9)</p> <p>P10-CGTGCGCGTCCAGCTCGACCA (SEQ ID NO:10)</p> <p>P11-TAGATGCGCTACAAGGACTCG (SEQ ID NO:11)</p> <p>P12-TCGGGATCCTCTACAAATGTGGTATGGCTG (SEQ ID NO:12)</p> <p>P14-ATCACGAGATCTCCACCATGGACTACAAAGACGATGACGATAAAATGGTGAGAGATAAAATG (SEQ ID NO:13)</p> <p>P15-ATAAGAATGCGGCCCGCGGATCCTTATGGTTTACATAGG (SEQ ID NO:14)</p> <p>P16-ATAAGAATCTCTGTTTATTGGTTAAGG (SEQ ID NO:15)</p> <p>P17-AACAGAGAATTCTTTATCATGTAGTCC (SEQ ID NO:16)</p> <p>P18-TCAGTTCGGCCGAATTACTCATAGCAATC (SEQ ID NO:17)</p> <p>P19-GTAATTGCGCCGAAGTGAAGCAACC (SEQ ID NO:18)</p> <p>P20-GGCACCAACCCCGGTGAACAG (SEQ ID NO:19)</p> <p>P21-GTATGGCTGATTATGATCTAG (SEQ ID NO:20)</p>

Please replace paragraph [0068] bridging pages 16 and 17 of the application with the following amended paragraph:

[0068] Fig. 1A depicts the A-I interaction. A conserved purine residue in the intron three nucleotides from the 3' cleavage site (molecule 1, R in box) must pair with a pyrimidine in the anticodon loop 6 nucleotides upstream of the 5' cleavage site (molecule 1, Y in box) to form the A-I (for anticodon-intron) interaction (Baldi, *et al.*, *supra*, 1992). BHB (molecule 2): Two bulges of three nucleotides each (where cleavage occurs) rigidly separated by four base pairs (Daniels, *et al.*, *supra*, 1985; Diener and Moore, *supra*, 1998). BHL (molecule 3): A three-nucleotide 3' site bulge, a four base-pair helix and a loop containing the 5' site. Pre-tRNA<sup>Archeuka</sup> (provided as SEQ ID NO:72 in the sequence listing) and its variants (molecule 4): The hybrid pre-tRNA molecule pre-tRNA<sup>Archeuka</sup> is a substrate for both the eukaryal and archaeal endonucleases. It consists of two regions derived from yeast pre-tRNA<sup>Phe</sup> (nucleotides (nt) 1-31 and not 38-76) joined by a 25 nt insert that corresponds to the BHB motif of the archaeal pre-tRNA<sup>Trp</sup>. It has a typical eukaryal mature domain with cleavage sites located at the prescribed distance from the reference elements and a correctly-positioned A-I base pair, all of which should ensure correct recognition by the eukaryal endonuclease when the enzyme operates in the mature-domain dependent mode. In addition, the presence of the BHB motif confers substrate characteristics that are recognizable by the eukaryal enzyme when it operates in the mature-domain independent mode. Fig. 1B depicts a substrate cleaved in both the mature-domain dependent and the mature domain independent modes. Products of digestion by the

*Xenopus* tRNA splicing endonuclease. Molecule 1: Pre-tRNA<sup>Archeuka</sup> 3bpΔas;  
molecule 2: Pre-tRNA<sup>Archeuka</sup> 3bpΔas, C56G.

Please replace the four lines immediately following paragraph [00170] on page 48 of the application with the following:

BGH1 (SEQ ID NO:21)

5'- ttg acg agt tct tctgaggggatccat tcctag agctcgctg atcagcc-3'

BGH2 (SEQ ID NO:22)

5'- agg acc tct aga aga tct gcc tgc tat tgt ctt ccc a-3'

Please replace the four lines immediately following paragraph [00172] on page 49 of the application with the following:

MJ1 (SEQ ID NO:23)

5'- CTC TGA CTG ACC GCG TTA CTC-3'

MJ2 (SEQ ID NO:24)

5'-TGC CGT TCT TGT CGA ACA CG-3'

Please replace Table 2 on page 53 of the application with the following amended Table 2:

TABLE 2	
Oligodeoxyribonucleotides	
P1	5'-GAGCTCAAGCTTCTGAATTCCTCGGTCTGACTCCAGAGGCTTACACCGGAGATATCACGACCGGTTGTGAGCAAGGGCGAG-3' (SEQ ID NO:25)
P2	5'-ATCAGGAGATCTCCACCATGGACTACAAAGACGATGACGATAAACTCGAGCTCAAGCTTCTGAATTC-3' (SEQ ID NO:26)
P3	5'-TCGGGATCCTCTACAAATGTGGTATGGCTG-3' (SEQ ID NO:27)
P4	5'-GCTTCGAATTCCTCGGTCTGACTTCTCCAGAGGCTTACACCGGAGAGATATCACGACCGGTTGTGA-3' (SEQ ID NO:28)
P5	5'-GCTTCGAATTCCTCGGTCTGACTCCAGAGGTAAGTAAACCGGAGATATCACGACCGGTTGTGA-3' (SEQ ID NO:29)
P6	5'-ATAAGAATGCGGCCGCGGTCGTGATCTCTCGGTGTAAGCCTCTGGAGTCACGACCGGGGACGGG-3' (SEQ ID NO:30)
P7	5'-ATAAGAATGCGGCCGCGGTCGTGATATCTTCTCGGTGTAAGCCTCTGGAGAAGTCACGACCGGGGACGGG-3' (SEQ ID NO:31)
P8	5'-TCACGACCGGGGACGGGGGCCAGACGGAGGGCGAGTCCTTGTAGCGCATCTACACATTGATCCTAGCAGA-3' (SEQ ID NO:32)
P9	5'-CGTCAGATCCGCTAGCGCTAC-3' (SEQ ID NO:33)
P10	5'-CGTCGCGCTCCAGCTCGACCA-3' (SEQ ID NO:34)
P11	5'-TAGATGCGCTACAAGGACTCG-3' (SEQ ID NO:35)
P12	5'-ATCAGGAGATCTCCACCATGGACTACAAAGACGATGACGATAAAATGGTGAGAGATAAAATG-3' (SEQ ID NO:36)
P13	5'-ATAAGAATGCGGCCGCGGATCCTTATGGTTTACATAGG-3' (SEQ ID NO:37)
P14	5'-ATAAGAATTCTCTGTTTATTTGGTTAAGG-3' (SEQ ID NO:38)
P15	5'-AACAGAGAATTCTTTATCATGTTAGCTCC-3' (SEQ ID NO:39)
P16	5'-TCAGTTGCGCCGAATTACTCATAGCAATC-3' (SEQ ID NO:40)
P17	5'-GTAATTGCGCCGAAGTGAAGTGAAGCAACC-3' (SEQ ID NO:41)
P18	5'-GGCACCACCCCGGTGAACAG-3' (SEQ ID NO:42)
P19	5'-GTATGGCTGATTATGATCTAG-3' (SEQ ID NO:43)
P20	5'-GACTCAGATCTCCACCATGGACTACAAAGACGATGACGATAAAGCCGGCAGAGATAAAATGGGCAAGAAGATCACCGGT-3' (SEQ ID NO:44)
P21	5'-GGCGCTCAGCTTGCTGATGCCGTTCTTGTGCAACACGATCACTCTGTCGCGCTCCAGCAGACCGGTGATCTTCTTGGCCAT-3' (SEQ ID NO:45)
P22	5'-GGCATCAGCAAGCTGAGCGCCAGGCACTATGGCAATGTGGAAGGCAATTTCTGAGCCTGAGCCTGGTGAAGCCCTGTAC-3' (SEQ ID NO:46)

P23 5'-TTCGAAGCTCAGGGGCTTGTTGTCTTATACTTCACCTCCAGCCAGCCCAGGTTGATCAGGTACAGGGCTTCCACCAGGCT-3'  
 (SEQ ID NO:47)  
 P24 5'-AACAAAGCCCCTGAGCTTCGAAGAGCTGTATGAATATGCCAGGAACGTGGAGGAAAGACTGTGTCTGAAGTACCTGGTGTAT-3'  
 (SEQ ID NO:48)  
 P25 5'-GAAGTCGGCGCCATACTTCAGGCCGGTCTTCAGGATATAGCCCCTGGTCCTCAGGTCCTTATACACCAGGTAATTCAGACA-3'  
 (SEQ ID NO:49)  
 P26 5'-CTGAAGTATGGCGCCGACTTCAGACTGTACGAAAGGGGCGCCAAACATCGACAAGGAGCACAGCGTGTATCTGGTGAAGGTG-3'  
 (SEQ ID NO:50)  
 P27 5'-GTGGGCCACTCTCACGAAGCCGGTCAGCTCGCTCAGCAGGAAGCTGCTGTCTTCAGGGAACACCTTACCAGATACACGCT-3'  
 (SEQ ID NO:51)  
 P28 5'-GGCTTCGTGAGAGTGGCCACAGCGTGAGAAAGAAGCTGCTGATCGCCATCGTGGACGCCGACGGCGACATCGTGTATTAC-3'  
 (SEQ ID NO:52)  
 P29 5'-  
 CCTCTACAAATGTGGTATGGCTGCTACGCGGCCGCGGATCCTTAAGGCTTCACATAGGTCATATTGTAATACAGATGTCGCCGTC-3'  
 (SEQ ID NO:53)  
 P30 5'-ATCGACAAGGAGTTCAGCGTGTATCTGGTG-3' (SEQ ID NO:54)  
 P31 5'-CAGATACACGCTGAATCCTTGTGATGTT-3' (SEQ ID NO:55)  
 P32 5'-GCCACAGCGTGAGACCTCAGCTGCTGATCGCCATC-3' (SEQ ID NO:56)  
 P33 5'-GATGGCGATCAGCAGCTGAGGTCTCAGCTGTGGGC-3' (SEQ ID NO:57)  
 P34 5'-TAGGGAAGCTTCGTGAGATCCGCTAGCGC-3' (SEQ ID NO:58)  
 P35 5'-AGAATGGCGCCGGGCCTTTCTTTATGTTTTGGCGTC-3' (SEQ ID NO:59)  
 P36 5'-  
 CGTCAGATCCGCTAGCGCTACCGGACTCAGATCAATTGCTGACTAGCCCGGAGATATCCTGGACCGGTTGAAGACGCCAAAAACATAA  
 AG-3' (SEQ ID NO:60)  
 P37 5'-GATCTGGATCCACCATGGTCCGGTCCAGGACTCCAGAGGGCTAGTCACTCGAGATCTA-3' (SEQ ID NO:61)  
 P38 5'-TAGATCTCGAGTGAAGTCCCTGAGTCTGGACCGGACCATGGTGGATCCAGATC-3' (SEQ ID NO:62)  
 P39 5'-GATCTGGATCCACCATGGTCCGGTCCAGGATATCTCCGGGCTAGTCACTCGAGATCTA-3' (SEQ ID NO:63)  
 P40 5'-TAGATCTCGAGTGAAGTCCCTGAGTCTGGACCGGACCATGGTGGATCCAGATC-3' (SEQ ID NO:64)  
 P41 5'-CCCACTGCTTACTGGCTTATCG-3' (SEQ ID NO:65)  
 P42 5'-CCCACTGCTTACTGGCTTATCG-3' (SEQ ID NO:66)  
 P43 5'-TGAGCTATTCCAGAAGTAGTG-3' (SEQ ID NO:67)  
 P44 5'-GGGAGTGGCACCTTCCAGGGTC-3' (SEQ ID NO:68)  
 P45 5'-GATATCAGGACCGGTTTCGTTTACTTTGACCAACAAGA-3' (SEQ ID NO:69)  
 P46 5'-TTCAGGCTGCGCAACTGTTGG-3' (SEQ ID NO:70)  
 P47 5'-TCTTGTGGTCAAAGTAAACGAAACCGGTCGTGATATC-3' (SEQ ID NO:71)